

水稻中一个谷胱甘肽转移酶基因的克隆、 表达和酶活性分析

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摘要: *OsGSTL1* 是位于水稻 3 号染色体上的一个 类谷胱甘肽转移酶基因, 由 8 个内含子和 9 个外显子组成, 编码一个由 243 个氨基酸组成的多肽链。将 *OsGSTL1* 克隆到酵母表达载体 pYTV 上, 转化大肠杆菌, 然后再转化酵母菌 PEP4。Western 印迹分析表明外源 *OsGSTL1* 基因在转基因酵母中表达, 分析半乳糖诱导表达的酵母粗提液的谷胱甘肽转移酶活性表明: 转基因酵母的谷胱甘肽转移酶较非转基因酵母和未诱导的酵母高, 说明 *OsGSTL1* 在转基因酵母中受半乳糖的诱导表达, 具有谷胱甘肽转移酶活性。

关键词: 谷胱甘肽转移酶; 水稻; Western 印迹分析; GST 活性

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Molecular Cloning, Expression and Activity Analysis of a Glutathione S-transferase in Rice^{*}

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Abstract: *OsGSTL1* is a glutathione S-transferase (GSTs, EC 2.5.1.18), lambda class gene in chromosome 3 of rice (*Oryza sativa* L.). It includes 8 introns and 9 exons, and encodes a protein of 243 amino acid residues. The *OsGSTL1* gene was cloned into pYTV vector and was transformed into yeast strain PEP4. Western blot analysis showed the exogenous *OsGSTL1* was expressed in transformed yeast. GST activity of crude extracts of yeast showed the *OsGSTL1* transgenic yeast had higher levels of GST activities than control yeasts, which demonstrate the *OsGSTL1* has glutathione S-transferase activity.

Key words: Glutathione S-transferase; *Oryza sativa* L.; Western blot analysis; GST activity

Glutathione S-transferases (GSTs, EC 2.5.1.18) are scavenging enzymes that detoxify cellular xenobiotics and toxins by catalyzing the conjugation of these substrates with a tripeptide glutathione. In animals, the first discovered GST was a result of its importance in the metabolism and detoxification of drugs in the 1960s. In plant, the first recognized GST was from maize as its GST activity to be responsible for conjugating the chloro-S-triazine atrazine with GSH in 1970.

GST activities, the corresponding enzymes and gene sequences have been identified in animals, plants and fungi (Wilce and Parker, 1994; Sheehan *et al.*, 2001).

The plant GST gene families are large and highly diverse. There are 48 members in *Arabidopsis*, over 25 in soybean (*Glycine max* L.), 42 in maize (*Zea mays* L.), and 59 in rice (*Oryza sativa* L.) (McGonigle *et al.*, 2000; Dixon *et al.*, 2002b; Wagner *et al.*,

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2002; Soranzo *et al.*, 2004). Based on protein homology and gene organization, plants GSTs have been categorized into five classes: phi (F), zeta (Z), tau (U), theta (T) and lambda (L) (Dixon *et al.*, 2002b).

The GST proteins have evolved by gene duplication to perform a range of functional roles using the tripeptide glutathione (GSH) as a cosubstrate or coenzyme. Classically, GSTs catalyze the transfer of the tripeptide glutathione (-glutamyl-cysteinyl-glycine; GSH) to a cosubstrate (R-X) containing a reactive electrophilic center to form a polar S-glutathionylated reaction product (R-SG) (Dixon *et al.*, 2002b). Some GSTs have been shown to have glutathione peroxidase activity, with the GSTs using glutathione to reduce organic hydroperoxides of fatty acids and nucleic acids to the corresponding monohydroxyalcohols (Cummins *et al.*, 1999; Dixon *et al.*, 2002a). And GSTs catalyze the isomerization of maleylacetoacetate to fumarylacetoacetate (Dixon *et al.*, 2000). Some GSTs also have ligand functions (Edwards *et al.*, 2000; Lederer and Boger, 2005). Some studies have also implicated GSTs as stress signaling proteins of some cell signaling pathways (Loyall *et al.*, 2000). GSTs also have function in cellular redox homeostasis or regulate apoptosis (Loyall *et al.*, 2000; Kampranis *et al.*, 2000; Dixon *et al.*, 2002a; Kunieda *et al.*, 2005).

In this report, *OsGSTL1* cDNA (AF 237487) was isolated from *Oryza sativa* cv. Zhonghua 11. Expression and activity of *OsGSTL1* in yeast strain PEP4 was studied. The results demonstrate *OsGSTL1* is a glutathione S-transferase (GSTs, EC 2.5.1.18).

Materials and methods

Culture of *Oryza sativa* cv. Zhonghua 11

The seeds of *Oryza sativa* cv. Zhonghua 11 were sterilized, soaked in water at 28 °C for 2 d, and then grown at 28 °C under a 16 h light dark photoperiod at an intensity of approximately 250 $\mu\text{E m}^{-2} \text{s}^{-1}$. Seven-day-old seedlings were collected and frozen in liquid nitrogen and stored at -80 °C.

Cloning and analysis of *OsGSTL1* gene

Total RNA from the leaves and roots of the seedlings were isolated with Trizol reagent (Gibco-BRL) (Vidmar *et al.*, 2000). Reverse transcriptions were performed with Oligo(dT) 18 as 3' primer and 1 μg RNA as templates.

The *OsGSTL1* sequence was amplified from cDNA by reverse transcriptase-PCR using Taq DNA polymerase and 30 cycles of 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 90 s, with the combinations of specific primers *OsGSTL1* f (5'-CACCACAAAATGGCCGAGCTGCAGCA-3') and *OsGSTL1* r (5'-GGCAACCTTAAGATGCGT-3'). A CACCACAAA sequence was introduced to the 5'-end of forward primer *OsGSTL1* f. This PCR product was cloned into pENTR D-TOPO vector (Invitrogen, USA) and sequenced. The nucleotide sequence and the putative amino acid sequence were analyzed with the DNAMAN and blastP (<http://www.ncbi.nlm.nih.gov>), respectively.

Expression of *OsGSTL1* gene in yeast strain PEP4

The *OsGSTL1* gene that had been cloned into pENTR D-TOPO vector was cloned into pYTV vector (Invitrogen, USA) by LR reaction, transformed into *E. coli* DH5 α , and then the correct expression vector was transformed into yeast strain PEP4. The *OsGSTL1* protein was expressed in PEP4 cell after 19 h incubation with SC-Ura medium including 2% glucose and 4 h induction with 2% galactose.

Western blot analysis

The crude protein extracts were prepared from the yeast. The yeast was homogenized in the Lysis buffer (50 mmol/L Tris pH 7.5, 1 mmol/L EDTA, 100 mmol/L NaCl, 0.1% TritonX-100, 1 mmol/L DTT, 1 mmol/L PMSF) and centrifuged at 4 °C. The supernatant was extracted with trichloroacetic acid and centrifuged at 4 °C, and then precipitate was washed twice with ethanol. The crude protein extracts was used to Western blot analysis and enzyme assay. Protein concentration in crude extracts was determined with the BioRad Protein Assay Kit (Bradford, 1976).

Proteins (15 μg) were separated in SDS-PAGE and transferred onto Immobilon membrane (Millipore, USA) with a semi-dry transfer cell (Millipore, USA). The recombinant *OsGSTL1* polypeptides expressed in yeast strain PEP4 was detected with an antibody raised against His-tag (Novagen, USA).

Activity assay of *OsGSTL1*

Protein concentration in crude extracts was adjusted to 0.5 mg/ml. GST activity was measured spectrophotometrically (Habig *et al.*, 1974; Takesawa *et al.*, 2002). One unit of activity was defined as the amount of enzyme required forming 1 μM product per minute at 30 °C.

Results

Isolation and bioinformatic analysis of *OsGSTL1*

OsGSTL1 gene was isolated from *Oryza sativa* cv. Zhonghua 11 by RT-PCR. It includes 8 introns and 9 exons, and encodes a protein of 243 amino acid residues.

OsGSTL1	-----MAAAAA-----PRSSGK
AtGSTL1	-----MALS-----PPKIFVE
AtGSTL2	MSVGLKVS AFLHPTLALSSRDVSLSSSSSSLYLDRKILRPGSGRRWCKSRRTPEILAVVE
Cla30	-----MAAAAA-----IASSTK
In2-1	-----MAAAAG-----PSSSVK
	. * .
OsGSTL1	EALPAALGSASEPPRLFDGTTRLYICYFCPFAQRAWIIRNFKGLQDKIELVGIDLQDKPA
AtGSTL1	DRQVP-LDATSDPPALFDGTTRLYISYTCPFAQRVWITRNLKGLQDEIKLVPIDLPNRPA
AtGSTL2	SSRVPELDSSSEPVQVFDGSTRLYISYTCPFAQRAWIARNYKGLQNKIELVPIDLKNRPA
Cla30	EVLPPALGAVSEPPPLFDGTTRLYICYICPFAQRAWVTRNCKGLQEEIKLVAINLEDKPA
In2-1	ESLPPALGSTSQPPPQVFDGTTRLYICYFCPFAQRAWVTRNLKGLQDKMELVAIDLQDKPA
	* . * * . ****. *****. * ***** * . ** ***** . ** * * . **
OsGSTL1	WYKEKVYEQGTVP SLEHNGKIMGESLDLIKYIDSHFEGPALLPEDPEKRQFADELIAYAN
AtGSTL1	WLKEKVNPA NKVPAL EHN GKITGESLDLIKYVDSNFDGPSLYPEDSAKREFGEELLKYVD
AtGSTL2	WYKEKVYSANKVPAL EHN RVLGESLDLIKYIDTNFEGPSLTPDGLEKQVVADELLSYTD
Cla30	WYKEKVYPQGTVP SLEHDGRVTGESLDLIKYIDTNFQGPALLPQDPAKRQFADELIAYAD
In2-1	WYKDKVYAQGTVP SLEHDSEVRGESLDLIRYIDSNFDGPALLPEDAAKRQFADELFASAN
	* *. ** . *. *** . *****. *. *, * ** * * . * . **
OsGSTL1	A-FTKALYSPLISKADLSAETVAALDKIEAALS KFGDGPFFLGQFSLVDIAYVTI IERI Q
AtGSTL1	ETFVKTVFGSFK--GDPVKETASAFDHVENALKKFDDGPFPLGELSVDIAYIPFIERFQ
AtGSTL2	S-FSKAVRSTLN--GTD TNAA DVAFDYIEQALS KFN EGPFPLGQFSLVDVAYAPFIERFR
Cla30	A-FTKALYSPLISQVAMSDEAVAALDKIEAALS KFS DGPFFLGQFSLVDIAYVTILERVQ
In2-1	A-FTKALYSPLL SHA AVSDEVVAALDKLEADLSKFDDGPFPLGQFSLADVAYVTILERVQ
	* *. . * * . * * * * . *****. ** *. ** . ** .
OsGSTL1	IYYSHIRKYEITNGRPNLEKFIEEINRIEAYTQTKNDPLYLLDLAKTHLKVA---
AtGSTL1	VFLDEVFKYEIIIGRPNLAAWIEQMNMVAYTQTKTDSEYVVNYFKRFM-----
AtGSTL2	LILSDVMNVDITSGRPNLALWIQEMNKIEAYTETRQDPQELVERYKRRVQAEARL
Cla30	IYYSNLRNRYEIAKDRPNLERYTEEMNKIEAYKQTKNVPLALLDAKRHLKIA---
In2-1	IYYSHLRNYDIAQGRPNLQEFIDEMNKIEAYAQTKN DPLFLDLAKSHLKIA---
	. . . * **** . . *. . ** *. . . * .

Fig . 1 Alignment of the peptide sequences of the OsGSTL1 with Lambda GSTs from *Arabidopsis* (AtGSTL1, AtGSTL2), maize (In2-1) and wheat (Cla30) .

The position of the putative active site cysteinyl residues are shaded gray . Residues that are identical and conserved between sequences are marked with asterisk and colon, respectively .

The analysis of protein function domain suggested OsGSTL1 has certain characteristics of glutathione-S-transferases . Alignment of the peptide sequences showed OsGSTL1 are a putative *Oryza Sativa* glutathione S-transferase, lambda class (OsGSTL) accordingly Dixon *et al.* (2002a) . Lambda class members have a cysteine residue in active center which distinguishes them from other class members which have a serine residue in same site (Fig.1) .

Expression of OsGSTL1 transgene in yeast

To investigate the function of OsGSTL1, the *OsGSTL1* gene was cloned into pYTV vector (Invitrogen, USA) and was transformed into yeast strain PEP4 . The

results of Western blot analysis showed the exogenous OsGSTL1 was expressed in transformed yeast, whereas no exogenous OsGSTL1 was detected in nontransformed yeast (Fig . 2) .

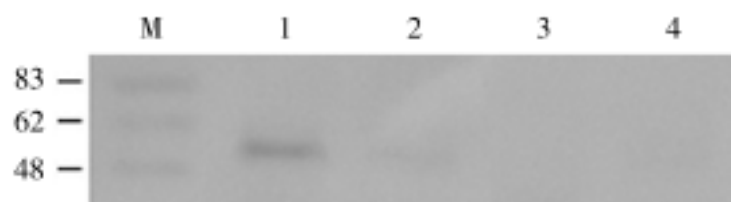


Fig . 2 Western-blot analysis of crude protein extract from OsGSTL1 transgenic yeast

M, protein marker; 1, OsGSTL1 transgenic yeast induced by galatose; 2, untreated OsGSTL1 transgenic yeast; 3, non-transgenic yeast induced by galatose; 4, untreated non-transgenic yeast

GST activity analysis of OsGSTL1

GST activity of crude extracts of yeast was measured with CDNB as the substrate . The findings showed the OsGSTL1 transgenic yeast that induced with 2 % galactose contains higher levels of GST activities than control yeasts . In yeast, transgenic strains with *OsGSTL1* have seven more times higher GSH conjugating activity than non-transgenic strain (Table 1) .

Table 1 Specific activities of GST in protein extracts from the transgenic yeast

Yeast strain	GST activity in CDNB ($\mu\text{mol min}^{-1}\text{mg}^{-1}\text{protein}$)	
	Non-treatment	Galactose treatment
Non-transformant	72.2 \pm 9.6	63.4 \pm 13.5
OsGSTL1 transgenic yeast	67.6 \pm 11.3	531.4 \pm 64.4

* The data are means of three independent experiments \pm SD

Discussion

GSTL1 proteins which have a cysteine redidue in active center are classified as lambda class GSTs, are presented in all eukaryotes from fungi to mammals (Dixon *et al.*, 2002a) . From an evolutionary perspective, it has been postulated that GSTL1 proteins were one of the most ancient genes . The structures of the *OsGSTL1* gene is very complex compared to those of other genes because their coding sequences include nine exons .

Some reports have shown lambda GSTs have no GSH conjugating . The maize In2-1 and soybean Gmin2-1 previously considered being not GSTs because of lack GSH conjugating activity towards CDNB (McGonigle *et al.*, 2000) . Arabidopsis lambda GSTs (AtGSTL1 and 2) have no GSH conjugating with standard substrates, but have activity of thiol transferases instead (Dixon *et al.*, 2002a) . From structure analysis, the serine residue in active center of other four classes (phi, zeta, tau, and theta) in plants which all have GSH conjugating activities is replaced by cysteine residue in lamda class and DHAR in plants . This substitution was considered to change the catalyzed property of lamda class and DHAR GSTs . However, yeast strains which transformed with *OsGSTL1* have elevated GSH conjugating activity, transgenic strains with *OsGSTL1* have seven more times higher GSH conjugating

activity than non-transgenic strain; it is difficult to believe that OsGSTL1 does not have GSH conjugating activity . We also noticed our measured values of GSH conjugating activity towards CDNB for OsGSTL1 were evidently higher than that of OsGSTF5 (Cho *et al.*, 2007), AtGSTU26 and AtGSTF9 (Nutricati *et al.*, 2006) . But the values of GSH conjugating activity for these GSTs were got by prokaryotic expression proteins, which were different from our results which were from eukaryotic system . The result of *Arabidopsis* lambda GSTs and maize in 2-1 which have been reported to have no GSH conjugating activity also was from that of prokaryotic expression proteins . The OsGSTL1 was also expressed in prokaryotic expression system, but the GSH conjugating activity was not fond (data not shown) . So, there are some differentials between lambda class GSTs which got from prokaryotic expression system and that from eukaryotic system . We speculate that the activities of GSTs may be affected by posttranslational modification such as phosphorylation which is lacked in prokaryotic system . Certainly, more evidence needs to be found to prove whether lambda class GSTs has GSH conjugating . However, our results provide valuable information to the understanding of the plant GST family especially lambda class GSTs .

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